

**DIARIED**

ID. 379.163.....

**PATENT COOPERATION TREATY**From the:  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Griffith Hack  
GPO Box 1285K  
MELBOURNE VIC 3001

GRIFFITH HACK

21 NOV 2005

1. Rec  
2.   
3. JSB**PCT**WRITTEN OPINION OF THE INTERNATIONAL  
PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 66)

Date of mailing  
(day/month/year)

17 NOV 2005

**REPLY DUE**within **TWO MONTHS**  
from the above date of mailing

Applicant's or agent's file reference

JSB:AJH:RMB:FP20705

International application No.

PCT/AU2004/001577

International filing date (day/month/year)

15 November 2004

Priority date (day/month/year)

14 November 2003

International Patent Classification (IPC) or both national classification and IPC

Int. Cl.<sup>7</sup> C12N 1/100, 1/20, 1/26, 1/38

Applicant

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al

1. ☒ The written opinion established by the International Searching Authority:☒ is ☐ is not

considered to be a written opinion of the International Preliminary Examining Authority.

2. This **SECOND** (second, etc.) opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

The applicant is hereby invited to reply to this opinion.

**When?** See the **Reply Due** date indicated above. However, the Australian Patent Office will not establish the Report before the earlier of (i) a response being filed, or (ii) one month before the **Final Date** by which the international preliminary examination report must be established. The Report will take into account any response (including amendments) filed before the Report is established. ~~If no response is filed by 1 month before the Final Date, the international preliminary examination report will be established on the basis of this opinion.~~

~~Applicants wishing to have the benefit of a further opinion (if needed) before the report is established should ensure that a response is filed at least 3 months before the Final Date by which the international preliminary examination report must be established.~~

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also** For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6.

4. ~~The FINAL DATE~~ by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 14 March 2006.

Name and mailing address of the IPEA/AU

AUSTRALIAN PATENT OFFICE  
PO BOX 200, WODEN ACT 2606, AUSTRALIA  
E-mail address: pct@ipaaustralia.gov.au  
Facsimile No. (02) 6285 3929

Authorized Officer

**GILLIAN ALLEN**

Telephone No. (02) 6283 2266

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WRITTEN OPINION OF THE  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

International application No.

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Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

- ☐ This opinion is based on a translation from the original language into the following language  
which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1 (b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this opinion has been established on the basis of *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed.")*:

- ☐ the international application as originally filed/furnished
- ☒ the description: pages 1-2, 4-62 as originally filed/furnished  
pages 3, received by this Authority on 25 August 2005 with the letter of 25 August 2005  
pages, received by this Authority on with the letter of
- ☒ the claims: pages 64-66, as originally filed/furnished  
pages, as amended (together with any statement) under Article 19,  
pages 63, received by this Authority on 25 August 2005 with the letter of 25 August 2005  
pages, received by this Authority on with the letter of
- ☒ the drawings: pages 1/17-17/17, as originally filed/furnished  
pages, received by this Authority on with the letter of  
pages, received by this Authority on with the letter of

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):

WRITTEN OPINION OF THE  
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**Box No. V**      **Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Claims 2-5, 9, 12, 13, 17, 18, 20, 21, 24 and 25	YES
	Claims 1, 6-8, 10, 11, 14-16, 19, 20, 23	NO
Inventive step (IS)	Claims	YES
	Claims 1-25	NO
Industrial applicability (IA)	Claims 1-25	YES
	Claims	NO

**Citations and explanations:**

**Citations**

- D1 Acha, V.; Meurens, M.; Naveau, H.; Agathos, S. N. Detoxification of a mixture of aliphatic chlorinated hydrocarbons in a fixed-bed bioreactor : continuous on-line monitoring via an attenuated total reflection-Fourier transform infrared sensor. Water Science and Technology (1999), 40(8), 41-47.
- D2 Stuart S L; Woods S L. Kinetic evidence for pentachlorophenol-dependent growth of a dehalogenating population in a pentachlorophenol- and acetate-fed methanogenic culture. Biotech and Bioeng, 1998. 57(4): 420-429.
- D3 Bellco CellTrol II Control Modules  
<http://www.bellcoglass.com/us/7803-81102.shtml>. 31 August 2003. <http://www.archive.org/> used to establish the publication date of the document.
- D4 BioNet Utility Tower (Single, Dual, or Quad)  
<http://www.broadleyjames.com/bionet-tower.html>. 2 October 2003. <http://www.archive.org/> used to establish the publication date of the document.

**New Citation**

- D5 Granato M et al. Biological treatment of a synthetic gold milling effluent. Environmental Pollution, 1996. 91(3): 343-350

**Novelty and Inventive Step**

It is noted that the new claims limit monitoring of the culture to measurement of metabolic indicators other than direct measurement of substrate. This aspect of the invention was not fully searched at the ISR. However, a new citation found during the original search has been provided. A copy is appended for your convenience.

Continued in Supplemental Box I

WRITTEN OPINION OF THE  
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**Box No. VIII** Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 19 is not clear.

I cannot determine what range of substrates the term "not a commonly metabolised substrate" might encompass. Nor can I determine at what point a substrate can be considered as "not commonly metabolised" in terms of any measurable quantity, such as the percentage of known microorganisms that metabolise the substrate.

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Supplemental Box 1

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Novelty and Inventive Step**

I cannot determine what aspect(s) of the invention could be assessed as providing inventive step.

As previously stated, neither enrichment cultures (either of single species or consortia) nor the use of continuous flow chemostats/bioreactors is new. It is noted that enrichment cultures may be monitored using any parameter used for any other type of microbial culture. It is common practice to determine parameters other than disappearance of substrate, for example, production of metabolites or biomass, or measurements of respiration such as ATP formation, are commonly used parameters. Online monitoring of various parameters of microbial metabolism or growth is not new, as indicated by D4 and D5. Moreover, if any parameter demanded new methods of measurement, the invention would lie only in the new measurement method. The use of output signals to regulate inputs into a biological culture is considered to be obvious experimental methodology. It is considered entirely obvious to preset temperature, pH and aeration (or lack thereof for anaerobic organisms) to predetermined values which the desired organisms require. Enrichment cultures are considered to inherently require selective pressure.

The applicants have argued that their methods comprise measurement of a common metabolic indicator such as O<sub>2</sub> to determine success of enrichment. However, claims 1-8, 10-25 are not limited to measurements of common metabolic indicators. It is noted that assessment of O<sub>2</sub> levels would not be a useful indicator for anaerobic cultures.

Therefore, none of the claims is considered inventive over normal microbiological methodology.

D5 discloses biological sludge microorganisms "acclimatisation" and the removal of free cyanide, thiocyanate and metalocyanides from synthetic gold milling effluent. The term "acclimatisation" is deemed synonymous with "enrichment". Fluid was fed into the reactor at a controlled rate comprising sewage (nutrient medium) and increasing concentrations of the gold milling effluent (test substrate). Reactor conditions were preset. Measurements were taken of chemical oxygen demand (COD), free cyanide, thiocyanate, copper, zinc and iron concentrations, and of mixed liquor volatile suspended solids (MLVSS). Of these, COD and MLVSS are indicators of microbial metabolism other than substrate concentration. It is apparent that oxygen is required to metabolise cyanide and thiocyanates. It is noted that the requirement that "the output based on the signal of the level of the metabolism indicator is a visual one" amounts to no more than a readable output on an analytical machine, or even a colour change, and is not considered to provide novelty. Also, the citation discloses MLVSS plotted against time (Fig 8). It is considered that cyanide and metal thiocyanates are "not commonly metabolised substances".

Claims 1, 6-8, 10, 11, 14-16, 19, 20-23 lack novelty and inventive step over D6.

It is considered that chemical oxygen demand could be readily determined using an oxygen electrode to provide an electronic signal that could be monitored online. The citation discloses requirement for high levels of dissolved oxygen, and that higher levels of dissolved oxygen compensate for decreased hydraulic retention time. Thus, it is obvious that measurements of dissolved oxygen levels could be used to regulate flow rates to optimise decontamination rates. The frequency of monitoring of microbial parameters would be readily determined by one skilled in the art. Moreover, if the parameter were measured electronically, monitoring could be at any interval, or continuous if desired. Isolation of the enriched organism(s) is obvious. Elevated temperature tolerance is a required parameter in many processes, eg microbial enzymes in washing detergents.

Claims 2-5, 9, 12, 13, 17, 18, 20, 21, 24 and 25 lack inventive step over D6.

Continued in supplemental box 2

WRITTEN OPINION OF THE  
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International Application No.

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**Supplemental Box 2**

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Novelty and Inventive Step**

D1, Acha et al, discloses acclimation (ie enrichment) of a biological consortium to a mixture of TCE, PCE, CT and HCB in a bioreactor. Although the consortium is disclosed as being pre-enriched, acclimation of the mixed bacterial culture to different environmental conditions can only be considered a form of enrichment. It is a selection for those bacteria able to successfully compete in the environmental conditions of the bioreactor. Figures 5 and 6 show that it requires some time of acclimation to "toxic shock" before dechlorination of the substrates becomes effective. The reaction is monitored on-line via an attenuated total reflection-fourier transform infrared sensor to track concentrations of the chlorinated substrates. Monitoring is continuous. The citation discloses flow of nutrients and chlorinated substrates through the bioreactor.

Even if it were accepted that this was not a true enrichment culture, it would be obvious to one skilled in the art that the methods of the citation could be used to monitor enrichment of dechlorinating microbial cultures, as it provides a way to measure rate of substrate disappearance, which is clearly a function of successful enrichment. It is also noted while that IR spectroscopy is used in the citation to measure chlorinated substrates, the technique can readily be used to monitor the majority of organic molecules.

Claims 1-5, 10-25 do not preclude measurement of products of metabolism of the substrate. It is particularly noted that monitoring for the level of products of metabolism of the substrate rather than for substrate level, is not considered to provide invention as the one is clearly a function of the other.

Therefore, claims 1-5, 10-25 are considered to lack invention over D1 in light of standard methods of the art.